

# EFFECTS OF BLOCKING HELPER T CELL INDUCTION IN VIVO WITH ANTI-Ia ANTIBODIES

## Possible Role of I-A/E Hybrid Molecules as Restriction Elements\*

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A large body of evidence indicates that the specificity of most T lymphocytes is not directed to antigen per se but to antigen associated with gene products of the major histocompatibility complex (MHC)<sup>1</sup> (1). In the case of T proliferative responses and helper T cells involved in T-B collaboration, antigen is presented to T cells by a class of macrophagelike accessory cells (henceforth termed macrophages) that express Ia determinants (2-8). In mice, these determinants are encoded by genes situated in the I-region of the H-2 complex. T cells respond to the association of antigen plus Ia determinants in an H-2-restricted fashion. Thus, in the case of normal homozygous mice, T cells have specificity for antigen plus self Ia determinants but generally (9-15), though not invariably (16, 17), fail to recognize antigen in association with allo-Ia antigens. Unlike homozygous mice, H-2-heterozygous mice contain two distinct subgroups of T cells, each of which is restricted by the H-2 (Ia) determinants of only one of the two parental strains (3, 7, 12-15, 18-21).

Studies in vitro have shown that T-macrophage interactions can be blocked by the addition of anti-Ia antibody during culture (15, 22-24). This paper examines the effects of stimulating T cells to antigen in the presence of anti-Ia antibody in vivo.

### Materials and Methods

*Mice.* (CBA × C57BL/6[B6])F<sub>1</sub> mice were purchased from Cumberland View Farms, Clinton, Tenn., and B10.A(4R) mice were a gift from Dr. Peter Doherty, Wistar Institute of Anatomy and Biology, Philadelphia, Pa. All other mice were obtained from The Jackson Laboratory, Bar Harbor, Maine.

*Cell Suspensions.* Purified populations of unprimed (CBA × B6)F<sub>1</sub> T cells were prepared by passing pooled mesenteric, axillary, inguinal, and cervical lymph node (LN) cells over nylon wool columns (8).

*Positive Selection.* Doses of  $\approx 7 \times 10^7$  purified (CBA × B6)F<sub>1</sub> T cells plus 0.5 ml of 25% sheep erythrocytes (SRC) were transferred intravenously into (CBA × B6)F<sub>1</sub> or B10.A(4R) mice exposed to 900 rad 2 d before. Anti-Ia<sup>k</sup> antibody (A.TH anti-A.TL antiserum or monoclonal anti-I-A<sup>k</sup> antibody [see below]) was injected intraperitoneally in a total dose of 2 ml/mouse, 1 ml being given 4-6 h before the injection of T cells plus SRC and another 1 ml injected the next day. At 5 d after T cell transfer, the donor T cells were recovered from the spleen plus

\* Supported by U. S. Public Health Service grants Ca-15822, AI-10961, AI-15393, and CA-09140.

<sup>1</sup> Abbreviations used in this paper: LN, lymph nodes; MHC, major histocompatibility complex; MLN, mesenteric lymph nodes; PFC, plaque-forming cells; SRC, sheep erythrocytes.

mesenteric lymph nodes (MLN) of the recipients for use as helper T cells. The mean percent recoveries of the injected T cells obtained from the spleen plus MLN of the host in a total of 12 experiments (7 for F<sub>1</sub> selection hosts and 5 for B10.A[4R] hosts) were: 33% for transfer of T cells plus SRC, 17% for T cells without SRC, 20% for T cells plus SRC plus A.TH anti-A.TL antiserum, and 17% for T cells plus SRC plus monoclonal anti-I-A<sup>k</sup> antibody (percentages expressed with respect to number of viable cells recovered). Serum taken from the anti-Ia<sup>k</sup>-injected selection hosts at the time of harvesting the donor T cells had no demonstrable cytotoxic activity.

*Anti-Ia<sup>k</sup> Reagents.* A.TH anti-A.TL (anti-Ia<sup>k</sup>) serum was obtained commercially from Cedarlane Laboratories Ltd., Hicksville, N. Y. Monoclonal anti-I-A<sup>k</sup> antibody was obtained from ascites fluid of (B6 × DBA/2)F<sub>1</sub> mice that sustain the growth of the mouse-mouse hybridoma 10-3.6 (IgG<sub>2a</sub>). This reagent detects a public I-A specificity (Ia.17) expressed by *k*, *f*, *r*, and *s* haplotypes but not by *b*, *d*, *p*, or *q* haplotypes (25). The hybridoma was kindly made available by the Herzenberg group, Stanford University School of Medicine, Stanford, Calif., and was forwarded to this laboratory by Dr. P. Murrack and Dr. J. Kappler, Rochester School of Medicine and Dentistry, Rochester, N. Y., after further cloning. Approximate titers of the two anti-Ia<sup>k</sup> reagents tested in the presence of guinea pig complement on spleen cells from CBA (*K<sup>k</sup>I-A<sup>k</sup>I-B<sup>k</sup>I-J<sup>k</sup>I-E<sup>k</sup>I-C<sup>k</sup>S<sup>k</sup>D<sup>k</sup>*) (*kkkkkkkk*), B10.A(4R) (*kkbbbbb*), B10.A(5R) (*bbkkddd*), and B10 (*bbbbbbb*) mice were: A.TH anti-A.TL: CBA 3<sup>6</sup>, 4R 3<sup>5</sup>, 5R 3<sup>5</sup>, B10 3<sup>3</sup> (indicating high activity for both I-A<sup>k</sup> and I-J/E<sup>k</sup> but only minimal activity for I-A<sup>b</sup>); hybridoma 10-3.6: CBA 3<sup>9</sup>, 4R 3<sup>9</sup>, 5R 3<sup>0</sup>, B10 3<sup>0</sup>. Cytotoxic indices of these anti-Ia<sup>k</sup> reagents tested on various Ia<sup>k</sup>-homozygous cell populations were: normal spleen 50–60%, anti-Thy-1.2-treated spleen 80–90%, normal LN 30–45%; for obscure reasons, the monoclonal anti-I-A<sup>k</sup> reagent showed plateau lysis of 20–30% on I-A<sup>k</sup>-heterozygous (CBA × B6)F<sub>1</sub> spleen cells (compared with 50–60% for A.TH anti-A.TL antiserum). With respect to T cells, both reagents had only minimal activity (<5% specific lysis) on nylon-wool-passed LN T cells. When tested on unfractionated LN, the sum of cytotoxic indices with the anti-Ia<sup>k</sup> reagents (30–45%) plus cytotoxic indices with anti-Thy-1.2 antibody (40–60%) was generally <100%; the addition of both anti-Ia<sup>k</sup> and anti-Thy-1.2 antibody produced 90–95% lysis. The activity of the anti-Ia<sup>k</sup> reagents for unfractionated LN T cells thus appeared to be minimal.

*T-B Collaboration.* As detailed elsewhere (8), small doses (0.4–0.8 × 10<sup>6</sup>) of the activated T cells recovered from the spleen plus MLN of the selection hosts were washed twice and transferred with B cells (SRC-primed spleen cells treated with anti-Thy-1.2 antibody and complement) and SRC into irradiated (700 rad) (CBA × B6)F<sub>1</sub> mice. IgM (direct) and IgG (indirect) plaque-forming cells (PFC) were measured in the spleen on day 7 posttransfer.

## Results

*Experimental Protocol.* The general approach was to activate purified (CBA × B6)F<sub>1</sub> (Ia<sup>k</sup> × Ia<sup>b</sup>) T cells to SRC in syngeneic irradiated F<sub>1</sub> mice in the presence of anti-Ia<sup>k</sup> antibody, harvest the activated T cells 5 d later, and then test their helper T function for parental strain B cells on further transfer. In initial experiments, A.TH anti-A.TL antiserum was used as a source of anti-Ia<sup>k</sup> antibodies. On the basis of cytotoxic testing with cells from various *H-2*-recombinant strains (Materials and Methods), this antiserum had high titered (3<sup>5</sup>–3<sup>6</sup>) activity (≈90% lysis) for T cell-depleted spleen cells that express either I-A<sup>k</sup> or I-E<sup>k</sup> subregion determinants, low activity on B10 spleen (3<sup>3</sup>), and little, if any, activity against purified (CBA × B6)F<sub>1</sub> T cells (≤5% lysis on nylon-wool-passed LN).

Experiments with A.TH anti-A.TL antiserum were designed arbitrarily as follows: (CBA × B6)F<sub>1</sub> mice were exposed to lethal irradiation (900 rad) and left for a period of 2 d, i.e., to allow time for clearance of radiosensitive Ia-positive cells such as B lymphocytes. On day 0, the mice (one single mouse/experiment) received 1 ml of unabsorbed A.TH anti-A.TL antiserum intraperitoneally and then, 4–6 h later, were injected intravenously with an inoculum of ≈7 × 10<sup>7</sup> nylon-wool purified (CBA ×

B6)F<sub>1</sub> LN T cells plus 0.5 ml of 25% SRC. 1 d later (day 1) the mice received another 1-ml intraperitoneal injection of A.TH anti-A.TL antiserum; control mice received no antiserum. On day 5, spleen and MLN cells were removed from the recipients (for number of T cells recovered, see Materials and Methods) to assess helper T function. Helper T function was measured by transferring small numbers ( $0.4\text{--}0.8 \times 10^6$ ) of the activated T cells plus SRC and parental strain or F<sub>1</sub> B cells (SRC-primed anti-Thy-1.2-treated spleen) into irradiated (CBA  $\times$  B6)F<sub>1</sub> mice for measurement of anti-SRC PFC.

*T Cell Selection in Presence of A.TH Anti-A.TL Antiserum.* The helper specificity of (CBA  $\times$  B6)F<sub>1</sub> T cells positively selected to SRC in an irradiated F<sub>1</sub> mouse given 2 ml of A.TH anti-A.TL (anti-Ia<sup>k</sup>) antiserum is shown in Table I. It is evident that the capacity of the activated F<sub>1</sub> T cells to stimulate anti-SRC PFC responses by CBA (Ia<sup>k</sup>) B cells was markedly reduced, both for IgM and IgG PFC production; these low responses were no higher than the responses given by unprimed T cells, i.e., by F<sub>1</sub> T cells passaged through irradiated F<sub>1</sub> mice in the absence of SRC. T cell help for B6 (Ia<sup>b</sup>) B cells, by contrast, was unaffected.

TABLE I  
*Positive Selection of (CBA  $\times$  B6)F<sub>1</sub> T Cells to SRC in Irradiated F<sub>1</sub> Mice in Presence of A.TH Anti-A.TL (Anti-Ia<sup>k</sup>) Antiserum\*: Selective Inhibition of Helper T Function for CBA B Cells*

Irradiated hosts used for positive selection of (CBA $\times$ B6)F <sub>1</sub> T cells to SRC	Antiserum added during selection	SRC added during selection	Dose of helper T cells	B cells for measuring helper T function‡	Anti-SRC PFC/spleen in irradiated (CBA $\times$ B6)F <sub>1</sub> mice	
					IgM	IgG
			$\times 10^6$			
(CBA $\times$ B6)F <sub>1</sub>	None	+	0.4	CBA	3,410 (1.14)§	13,690 (1.09)
			0.8	CBA	6,020 (1.40)	27,980 (1.16)
			0.4	B6	2,760 (1.64)	4,660 (1.25)
			0.8	B6	13,160 (1.09)	10,440 (1.39)
(CBA $\times$ B6)F <sub>1</sub>	A.TH anti-A.TL (anti-Ia <sup>k</sup> )	+	0.4	CBA	380 (2.32)	50 (2.20)
			0.8	CBA	780 (1.18)	1,230 (1.19)
			0.4	B6	3,480 (1.28)	6,750 (1.27)
			0.8	B6	4,750 (1.38)	10,520 (1.13)
(CBA $\times$ B6)F <sub>1</sub>	None	-	0.4	CBA	60 (1.26)	290 (1.20)
			0.8	CBA	180 (1.41)	1,710 (1.28)
			0.4	B6	110 (1.26)	0
			0.8	B6	90 (1.41)	570 (1.28)

\* As described in text, irradiated (CBA  $\times$  B6)F<sub>1</sub> mice exposed to 900 rad 2 d before were given  $7 \times 10^7$  nylon-wool-passed (CBA  $\times$  B6)F<sub>1</sub> LN T cells (93% Thy-1.2-positive) intravenously plus 0.5 ml of 25% SRC. The single recipient of A.TH anti-A.TL antiserum was given the serum in divided doses intraperitoneally, 1 ml 4 h before the injection of T cells plus SRC and 1 ml on the next day. Activated helper T cells were recovered from spleen plus MLN on day 5 after T cell transfer. As controls, irradiated F<sub>1</sub> mice (2 mice/group) received T cells plus SRC but not antiserum, or T cells alone.

‡ Helper T cells plus SRC (0.1 ml of 5%) were transferred together with CBA or B6 B cells (anti-Thy-1.2-treated SRC-primed spleen) into irradiated (CBA  $\times$  B6)F<sub>1</sub> mice for measurement of splenic PFC on day 7.

§ Geometric mean of data from 3-4 mice/group. Number in parenthesis refers to value by which mean is multiplied or divided to give upper and lower limits, respectively, of SE. Background values given by B cells transferred without T cells have been subtracted. These values ranged between 0 and 470 PFC/spleen. PFC for T cells transferred without B cells were <100 PFC/spleen.

To map the restriction in helper T function, T-B collaboration was examined with B cells from the B10 congenic lines. As shown in Table II, (CBA × B6)F<sub>1</sub> T cells selected to SRC in the presence of A.TH anti-A.TL serum (A.TH anti-A.TL F<sub>1</sub> T cells) gave only minimal PFC responses with B10.BR (kkkkkkkk) and B10.A(4R) (kkbbbbbb) B cells but collaborated well with B10 (bbbbbbbb) B cells. Suppression seemed to be an unlikely explanation for the restriction because good responses were observed with (B10 × B10.BR)F<sub>1</sub> B cells. Likewise, addition of the A.TH anti-A.TL F<sub>1</sub> T cells to the control F<sub>1</sub> T cells did not cause demonstrable suppression. Finally, five-fold higher doses of A.TH anti-A.TL F<sub>1</sub> T cells did give significant responses with B10.BR B cells. Thus, these findings are against the possibility that carry-over of anti-Ia<sup>k</sup> antibody with the helper T cells was responsible for the restriction in helper function. In this respect it should be mentioned that, after harvest, the activated T cells were washed twice before transfer with B cells. Moreover, at the time the activated T cells were removed, the serum of the selection hosts had no detectable anti-Ia<sup>k</sup> activity (Materials and Methods).

The data shown in Tables I and II are representative of six experiments. In all experiments the reduction in the IgG response with homozygous Ia<sup>k</sup>-bearing B cells was >85%. For IgM responses the reduction was less marked but was usually >70%. There was generally no change in the response with B10 cells: a mild reduction was observed on occasions (Table III) but this was countered by increased B responses in other experiments (Table II).

As a working hypothesis, the above findings were taken to imply that, on transfer to irradiated (CBA × B6)F<sub>1</sub> mice, antibodies in the A.TH anti-A.TL antiserum bound to the Ia<sup>k</sup> determinants on the host macrophages. This binding blocked the capacity of the macrophages to present antigen to the Ia<sup>k</sup>-restricted subgroup of F<sub>1</sub> T cells, but did not interfere demonstrably with presentation to the Ia<sup>b</sup>-restricted subgroup.

TABLE II  
*Helper Function of (CBA × B6)F<sub>1</sub> T Cells Activated to SRC in Irradiated F<sub>1</sub> Mice in Presence of A.TH Anti-A.TL Antiserum\*: T-B Collaboration with B10.BR, B10.A(4R), and B10 B Cells*

Group	Irradiated hosts used for positive selection of (CBA × B6)F <sub>1</sub> T cells to SRC	Antiserum added during selection	Dose of helper T cells	B cells for measuring helper T function*	H-2 haplo-	Anti-SRC PFC/spleen in irradiated (CBA × B6)F <sub>1</sub> mice	
					type of B cells	IgM	IgG
					$K \frac{I}{A B J F C} S D$		
A	(CBA × B6)F <sub>1</sub>	None	× 10 <sup>6</sup> 0.7	B10.BR	<u>kkkkkkkk</u>	10,300 (1.22)*	41,190 (1.11)
				B10.A(4R)	<u>kkbbbbb</u>	9,380 (1.14)	35,610 (1.17)
				B10	<u>bbbbbbb</u>	12,090 (1.16)	20,730 (1.11)
				(B10 × B10.BR)F <sub>1</sub>		8,800 (1.16)	28,260 (1.32)
B	(CBA × B6)F <sub>1</sub>	A.TH anti-A.TL (anti-Ia <sup>k</sup> )	0.7	B10.BR	<u>kkkkkkkk</u>	2,760 (1.36)	4,140 (1.24)
				B10.A(4R)	<u>kkbbbbb</u>	840 (1.15)	560 (1.91)
				B10	<u>bbbbbbb</u>	25,780 (1.24)	55,390 (1.20)
				(B10 × B10.BR)F <sub>1</sub>		6,670 (1.17)	33,440 (1.11)
Group A + group B T cells			0.7 of each	B10.BR	<u>kkkkkkkk</u>	8,200 (1.21)	39,470 (1.27)
Group B T cells			3.5	B10.BR	<u>kkkkkkkk</u>	4,180 (1.13)	28,570 (1.12)

\* As for Table I. Subtracted background values for B cells transferred without T cells ranged from 360 to 1,280 PFC/spleen. T cells alone gave < 100 PFC/spleen.

TABLE III  
*Apparent Failure of Monoclonal Anti-I-A<sup>k</sup> Antibodies to Block Positive Selection of (CBA × B6)F<sub>1</sub> T Cells\**

Irradiated hosts used for positive selection of (CBA × B6)F <sub>1</sub> T cells to SRC	Antibodies added during selection	SRC added during selection	B cells for measuring helper T function*	Anti-SRC PFC/spleen in irradiated (CBA × B6)F <sub>1</sub> mice	
				IgM	IgG
(CBA × B6)F <sub>1</sub>	None	+	B10.BR	8,930 (1.34)*	30,380 (1.36)
			B10	19,850 (1.41)	44,790 (1.32)
(CBA × B6)F <sub>1</sub>	None	-	B10.BR	310 (1.39)	1,100 (1.38)
			B10	780 (1.91)	0
(CBA × B6)F <sub>1</sub>	A.TH anti-A.TL (anti-Ia <sup>k</sup> antiserum)	+	B10.BR	2,850 (1.33)	2,830 (1.52)
			B10	11,780 (1.67)	23,790 (1.69)
(CBA × B6)F <sub>1</sub>	Monoclonal anti-I-A <sup>k</sup> (10-3.6 ascites fluid)	+	B10.BR	22,980 (1.07)	32,820 (1.12)
			B10	25,310 (1.57)	37,830 (1.44)

\* As for Table I and II. Helper T cells transferred in dose of  $0.7 \times 10^6$  cells. Subtracted background values for B cells transferred without T cells ranged from 50 to 1,000 PFC/spleen, T cells alone gave <400 PFC/spleen.

Because restrictions affecting T-macrophage interactions and T-B collaboration have been mapped to the *K/I-A* subregion of the *H-2* complex (Discussion), it was reasoned that the blocking activity of the A.TH anti-A.TL antiserum was mediated by antibodies with specificity for I-A<sup>k</sup> determinants rather than for I-E<sup>k</sup> determinants. If so, purified anti-I-A<sup>k</sup> antibodies would be expected to have comparable blocking activity.

*T Cell Selection in Presence of Monoclonal Anti-I-A<sup>k</sup> Antibody.* Analogous experiments in which (CBA × B6)F<sub>1</sub> T cells were selected to SRC in irradiated F<sub>1</sub> mice in the presence of monoclonal anti-I-A<sup>k</sup> antibody (2 ml of ascites fluid from mice that bear the hybridoma 10-3.6) are shown in Table III. This antibody (IgG<sub>2a</sub>) had high cytotoxic activity for homozygous I-A<sup>k</sup>-bearing B cells but no demonstrable activity for B10 B cells or (CBA × B6)F<sub>1</sub> T cells (Materials and Methods). It is evident from Table III that, in contrast to A.TH anti-A.TL antiserum, selection in the presence of the anti-I-A<sup>k</sup> antibody failed to inhibit the helper T response for B10.BR B cells; this applied to both IgM and IgG responses and was observed in six separate experiments.

Various trivial explanations could be invoked to account for the apparent failure of the monoclonal anti-I-A<sup>k</sup> antibodies to block selection: for example, the antibodies might have had poor homing capacity, a short survival time, or inadequate binding avidity, etc. For completeness, however, it was decided to repeat the experiments and include both B10.A(4R) and B10.BR B cells for measuring helper T function. The surprising finding (Table IV) was that, despite the high levels of help provided for B10.BR B cells, F<sub>1</sub> T cells selected in the presence of monoclonal anti-I-A<sup>k</sup> antibody gave only minimal responses with B10.A(4R) B cells; three other experiments gave similar data.

*T Cell Selection in B10.A(4R) Mice.* The above findings are clearly difficult to reconcile with the generally accepted view that helper T cells are restricted entirely by the *I-A* subregion (Discussion). Nevertheless, on a priori grounds, the data in Table

TABLE IV  
 Helper Function of (CBA × B6)<sub>F</sub><sub>1</sub> T Cells Activated to SRC in Irradiated F<sub>1</sub> Mice in Presence of Monoclonal Anti-I-A<sup>k</sup> Antibody\*: Reduction of Helper T Function for B10.A(4R) B Cells

Irradiated hosts used for positive selection of (CBA × B6) <sub>F</sub> <sub>1</sub> T cells to SRC	Antibodies added during selection	Dose of helper T cells	B cells*	H-2 haplo-	Anti-SRC PFC/spleen in irradiated (CBA × B6) <sub>F</sub> <sub>1</sub> mice	
				type	IgM	IgG
				$K \frac{I}{ABJEC} SD$		
(CBA × B6) <sub>F</sub> <sub>1</sub>	None	0.35	B10.BR	$\overline{k k k k k k k k}$	12,100 (1.28)*	32,280 (1.03)
		0.70	B10.BR	$\overline{k k k k k k k k}$	17,500 (1.09)	58,150 (1.11)
		0.35	B10.A(4R)	$\overline{k k b b b b b b}$	15,820 (1.20)	52,480 (1.04)
		0.70	B10.A(4R)	$\overline{k k b b b b b b}$	24,020 (1.04)	57,680 (1.07)
		0.70	B10	$\overline{b b b b b b b b}$	4,700 (1.16)	15,650 (1.12)
(CBA × B6) <sub>F</sub> <sub>1</sub>	Monoclonal Anti-I-A <sup>k</sup>	0.35	B10.BR	$\overline{k k k k k k k k}$	9,120 (1.31)	37,470 (1.09)
		0.70	B10.BR	$\overline{k k k k k k k k}$	13,450 (1.22)	52,550 (1.15)
		0.35	B10.A(4R)	$\overline{k k b b b b b b}$	1,020 (1.40)	1,870 (1.27)
		0.70	B10.A(4R)	$\overline{k k b b b b b b}$	2,660 (1.72)	2,490 (1.64)
		0.70	B10	$\overline{b b b b b b b b}$	4,830 (1.16)	17,370 (1.04)

\* As for Tables I-III. Subtracted background values for B cells transferred without T cells ranged from 330 to 1,980 PFC/spleen. T cells alone gave <300 PFC/spleen.

IV might be taken to imply that, in addition to the I-A<sup>k</sup>-restricted T cells, there also exist T cells restricted to I-a<sup>k</sup> molecules encoded wholly or in part by genes mapping to the right of I-A, e.g., in the I-E subregion. T cells restricted to these molecules would be able to interact with B10.BR (I-E<sup>k</sup>) B cells but not collaborate with B10.A(4R) (I-E<sup>b</sup>) B cells. By the same token, positive selection of these E<sup>k</sup>-restricted T cells would occur in (CBA × B6)<sub>F</sub><sub>1</sub> (I-E<sup>k/b</sup>) mice but not in B10.A(4R) mice. According to this interpretation, the capacity of (CBA × B6)<sub>F</sub><sub>1</sub> T cells to collaborate with B10.BR or B10.A(4R) B cells after selection to SRC in B10.A(4R) mice would be mediated entirely by the I-A<sup>k</sup>-restricted subgroup of T cells. If so, adding monoclonal anti-I-A<sup>k</sup> antibody during selection in B10.A(4R) mice should block helper function for both B10.BR and B10.A(4R) B cells.

To test this prediction, (CBA × B6)<sub>F</sub><sub>1</sub> T cells were positively selected to SRC in irradiated B10.A(4R) mice in the presence of either A.TH anti-A.TL antiserum, monoclonal anti-I-A<sup>k</sup> antibody, or no antibody (Table V). As reported previously (14), the control F<sub>1</sub> T cells activated in B10.A(4R) mice in the absence of antibody collaborated well with I-A<sup>k</sup>-bearing B10.BR, B10.A(4R), and (B10 × B10.BR)<sub>F</sub><sub>1</sub> B cells but did not stimulate B10 (I-A<sup>b</sup>) B cells. By contrast, F<sub>1</sub> T cells activated in the presence of either the broad spectrum anti-I-a<sup>k</sup> reagent (A.TH anti-A.TL) or the monoclonal anti-I-A<sup>k</sup> antibody failed to stimulate any of the four groups of B cells. To exclude the possibility that the T cells in this situation had been inactivated nonspecifically, selection was studied in irradiated B10.A(4R) mice injected with T cell-depleted irradiated B10 spleen cells, i.e., a source of I-A<sup>b</sup>-bearing antigen-presenting cells; these cells were given 1 h before the injection of F<sub>1</sub> T cells plus SRC (Table VI, footnote). As shown in Table VI, F<sub>1</sub> T cells selected to SRC in B10-spleen-injected B10.A(4R) mice in the presence of anti-I-A<sup>k</sup> antibody provided effective help for B10 B cells while remaining unresponsive for both B10.BR and B10.A(4R) B cells.

TABLE V  
 Helper Function of (CBA × B6)<sub>F</sub><sub>1</sub> T Cells Activated to SRC in Irradiated B10.A(4R) Mice in the Presence of Monoclonal Anti-I-A<sup>k</sup> Antibody\*

Irradiated hosts used for positive selection of (CBA × B6) <sub>F</sub> <sub>1</sub> T cells to SRC	Antibodies added during selection	B cells	H-2 haplo-type*	Anti-SRC PFC/spleen in irradiated (CBA × B6) <sub>F</sub> <sub>1</sub> mice	
			$K \frac{I}{A B J E C} S D$	IgM	IgG
B10.A(4R)	None	B10.BR	<u>k k k k k k k k</u>	6,600 (1.30)*	22,600 (1.18)
		B10.A(4R)	<u>k k b b b b b b</u>	17,650 (1.71)	15,640 (1.09)
		B10	<u>b b b b b b b b</u>	310 (1.81)	0‡
		(B10 × B10.BR) <sub>F</sub> <sub>1</sub>		5,220 (1.27)	21,640 (1.12)
B10.A(4R)	Monoclonal anti-I-A <sup>k</sup>	B10.BR	<u>k k k k k k k k</u>	300 (1.04)	110 (1.30)
		B10.A(4R)	<u>k k b b b b b b</u>	300 (1.30)	0
		B10	<u>b b b b b b b b</u>	420 (1.82)	570 (1.57)‡
		(B10 × B10.BR) <sub>F</sub> <sub>1</sub>		320 (1.46)	280 (1.23)
B10.A(4R)	A.TH anti-A.TL (anti-Ia <sup>k</sup> )	B10.BR	<u>k k k k k k k k</u>	920 (1.25)	1,030 (1.19)
		B10.A(4R)	<u>k k b b b b b b</u>	760 (1.42)	490 (1.30)
		B10	<u>b b b b b b b b</u>	500 (1.11)	240 (1.25)‡

\* As for Table IV, except that irradiated B10.A(4R) mice were used for positive selection. Helper T cells transferred in a dose of  $0.7 \times 10^6$  cells. Subtracted background values for B cells transferred without T cells were in the range of 50–250 PFC/spleen. T cells alone gave <100 PFC/spleen.

‡ These B cells gave high responses (>10,000 PFC/spleen) when transferred with  $4 \times 10^6$  nylon-wool-passed SRC-primed (CBA × B6)<sub>F</sub><sub>1</sub> T cells.

*Role of the I-E<sup>k</sup> Region in Restricting Helper T Function.* To seek direct information on whether I-E<sup>k</sup> molecules can act as restriction sites, (A/J (kkkkkddd) × B6)<sub>F</sub><sub>1</sub> T cells were positively selected to SRC in irradiated B10.A (kkkkkddd) or B10.A(5R) (bbbkddd) mice and then tested for helper T function with B cells from these two strains and also with B10 B Cells. As shown in Table VII, F<sub>1</sub> T cells selected to SRC in B10.A (I-A<sup>k</sup>, I-E<sup>k</sup>) mice collaborated well with B10.A B cells but did not give demonstrable helper activity for B10.A(5R) (I-A<sup>b</sup>, I-E<sup>k</sup>) or B10 B cells. Likewise, selection in B10.A(5R) mice failed to provide help for B10.A B cells but generated high levels of help for both B10.A(5R) and B10 B cells. These findings thus corroborate previous findings of other workers (Discussion) that I-E (or I-J) determinants per se do not act as restriction sites.

## Discussion

Before attempting to interpret the confusing pattern of results considered above, it may be useful to briefly review the evidence that the restrictions affecting helper T cells map in the I-A subregion. The importance of this subregion is apparent from the repeated finding that, although homozygous T cells generally fail to interact with macrophages or B cells across whole H-2 haplotype barriers, H-2 compatibility (or H-2 sharing) limited only to the I-A (or K/I-A) subregion leads to effective cell interaction (11, 13, 14, 26, 27). By contrast, total mismatching at the I-A subregion, but with a sharing of determinants encoded by the I-B through I-C subregions or parts thereof,

TABLE VI

*Helper Function of (CBA × B6)F<sub>1</sub> T Cells Activated to SRC in the Presence of Monoclonal Anti-I-A<sup>k</sup> Antibody in B10.A(4R) Mice Supplemented with Irradiated B10 Spleen\**

Group	Irradiated hosts used for positive selection of (CBA × B6)F <sub>1</sub> T cells to SRC	Antibodies added during selection	B cells*	Anti-SRC PFC/spleen in irradiated (CBA × B6)F <sub>1</sub> mice	
				IgM	IgG
A	B10.A(4R)	None	B10.BR	17,380 (1.21)*	35,470 (1.17)
			B10.A(4R)	22,460 (1.18)	38,990 (1.20)
			B10	380 (1.49)	700 (1.36)
B	B10.A(4R) injected with 1,200 rad anti- $\theta$ -B10 spleen‡	None	B10.BR	12,640 (1.12)	29,830 (1.13)
			B10.A(4R)	16,840 (1.10)	25,760 (1.20)
			B10	2,890 (1.13)	9,630 (1.01)
C	B10.A(4R) injected with 1,200 rad anti- $\theta$ -B10 spleen‡	Monoclonal anti-I-A <sup>k</sup>	B10.BR	500 (1.88)	230 (1.35)
			B10.A(4R)	2,170 (1.39)	940 (1.46)
			B10	7,360 (1.13)	10,350 (1.13)
Group B + Group C T cells (0.6 × 10 <sup>6</sup> of each)			B10.BR	15,120 (1.22)	33,600 (1.17)

\* As for Table IV. Helper T cells transferred in a dose of 0.6 × 10<sup>6</sup> cells. Subtracted background values for B cells transferred without T cells ranged from 50 to 360 PFC/spleen.

‡ 5 × 10<sup>7</sup> viable B10 spleen cells pretreated with anti-Thy-1.2 serum plus complement (anti- $\theta$ -spleen) and then exposed to 1,200 rad before intravenous injection into irradiated B10.A(4R) mice plus SRC; F<sub>1</sub> T cells given 1 h later.

does not lead to successful interaction. In the case of heterozygous T cells, this is exemplified by the helper specificity of (CBA × B6)F<sub>1</sub> T cells after positive selection to SRC in irradiated B10.A(4R) (*kkbbbbb*) mice. F<sub>1</sub> T cells activated in these mice give high levels of help for both B10.BR and B10.A(4R) B cells but, significantly, do not interact with B10 B cells (Table V); likewise, F<sub>1</sub> T cells selected to SRC in B10 mice do not have demonstrable helper activity for B10.A(4R) B cells (14). In this situation, therefore, the *I-B* through *H-2D* region of the *H-2* complex does not play a discernible role in controlling T cell function, either at the level of T cell selection or during T-B collaboration.

It is important to point out that B10 and B10.A(4R) mice do not express a detectable I-E product on the cell surface (28). Hence, to examine the possible role of the *I-E* subregion in controlling cell interactions one must turn to a haplotype where an I-E product is expressed, e.g., to the *H-2<sup>k</sup>* or *H-2<sup>a</sup>* (*kkkkkddd*) (A/J, B10.A) haplotype. In this respect, it is significant that activation of normal (A/J × B6)F<sub>1</sub> T cells to SRC in irradiated B10.A mice generated T cell help for B10.A (*kkkkkddd*) B cells but not for I-E-compatible B10.A(5R) (*bbkkkddd*) B cells (Table VII). Thus, this finding argues that I-E<sup>k</sup> determinants per se do not act as restriction elements. A number of other groups have reached a similar conclusion (11, 13, 26).<sup>2</sup>

Collectively, the above findings would seem to constitute strong evidence that determinants mapping to the right of the *I-A* subregion do not play a discernible role in controlling cell interactions. How, then, does one account for the finding that

<sup>2</sup> Low (10–25% of normal) collaborative responses with H-2 compatibility limited to the *I-E/C* subregion has been reported by Martinez et al. (29) for a system in which hapten-specific T cells stimulate hapten-modified B cells polyclonally.



TABLE VII  
*Helper T Specificity of (A/J × B6)F<sub>1</sub> T Cells Positively Selected to SRC in Irradiated B10.A, B10.A(5R), or B10 Mice*

Irradiated hosts used for positive selection of (A/J × B6)F <sub>1</sub> T cells to SRC*	SRC added during selection	B cells‡	H-2 haplo-type	Anti-SRC PFC/spleen in irradiated (A/J × B6)F <sub>1</sub> mice	
			$K \frac{I}{A B J E C} S D$	IgM	IgG
B10.A	+	B10.A	<i>k k k k k d d d</i>	9,860 (1.43)§	41,650 (1.15)
		B10.A(5R)	<i>b b b k k d d d</i>	90 (1.22)	100 (1.44)
		B10	<i>b b b b b b b b</i>	0	60 (1.26)
B10.A	-	B10.A	<i>k k k k k d d d</i>	360 (1.47)	0
		B10.A(5R)	<i>b b b k k d d d</i>	50 (1.49)	140 (1.65)
		B10	<i>b b b b b b b b</i>	0	170 (1.12)
B10.A(5R)	+	B10.A	<i>k k k k k d d d</i>	840 (1.22)	100 (2.05)
		B10.A(5R)	<i>b b b k k d d d</i>	8,760 (1.23)	41,120 (1.05)
		B10	<i>b b b b b b b b</i>	3,780 (1.28)	21,070 (1.36)
B10	+	B10.A	<i>k k k k k d d d</i>	160 (1.44)	170 (1.43)
		B10.A(5R)	<i>b b b k k d d d</i>	10,470 (1.19)	29,600 (1.42)
		B10	<i>b b b b b b b b</i>	5,740 (1.28)	21,270 (1.13)

\*  $65 \times 10^6$  purified (A/J × B6)F<sub>1</sub> T cells ± SRC given to irradiated (850 rad 1 d before) selection hosts of various strains. Donor cells recovered from spleen plus MLN on day 5 and used as helper T cells in doses of  $0.7 \times 10^6$  viable cells.

‡ SRC-primed B cells transferred in doses of  $10^7$  for B10.A,  $8 \times 10^6$  for B10.A(5R), and  $12 \times 10^6$  for B10.

§ Subtracted background values for B cells transferred without T cells ranged from 0 to 270 PFC/spleen. T cells alone gave <50 PFC/spleen.

selection of (CBA × B6)F<sub>1</sub> T cells to SRC in irradiated F<sub>1</sub> mice in the presence of anti-I-A<sup>k</sup> antibody suppressed the generation of help for B10.A(4R) B cells but not for B10.BR B cells? Such a finding implies that *I*-region genes telomeric to the *I-A* subregion do influence cell interactions. In speculating on the possible function of these genes, the recent evidence on the biochemistry of *I*-region gene products is particularly germane. It is well accepted that classic I-A molecules consist of two chains, α and β, both of which are encoded by genes situated in the *I-A* subregion (28, 30). In addition, Jones et al. (31) and others (32, 33) have demonstrated the existence of a different set of 2-chain molecules encoded by two separated genes: one gene (*E<sub>α</sub>*) maps in the *I-E* subregion and codes for the relatively nonpolymorphic α-chain, whereas the other gene (*A<sub>E</sub>*) is situated in the *I-A* subregion and encodes the highly polymorphic β-chain (*E<sub>β</sub>*); these A/E hybrid molecules can be formed by either *cis* or *trans* chain association.

In the case of antigens not under demonstrable *Ir* gene control, there is no direct evidence that E<sub>β</sub>-E<sub>α</sub> A/E hybrid molecules can act as restricting elements for cell interactions. Nevertheless, Schwartz et al. (34, 35) argue persuasively that such hybrid molecules are of critical importance in controlling T cell responses to antigens under double *Ir* gene control such as GLφ (36); responsiveness to these antigens is controlled by two distinct genes that map in the *I-A* and *I-E* subregions, respectively. It is also of interest that hybrid molecules can act as alloantigens, both for the mixed-lympho-

cyte reaction (37) and cell-mediated lympholysis (K. Fischer-Lindahl and B. Hausman. Personal communication.).

Based on the supposition that  $E_{\beta}$ - $E_{\alpha}$  hybrid molecules can act as restriction elements for antigens not under *Ir* gene control, the present data can be explained in terms of the following assumptions:

(a) (CBA  $\times$  B6) $F_1$  T cells comprise at least four subgroups of T cells. Two subgroups are restricted by the *I-A*-encoded determinants of CBA ( $A_{\beta}^k$ - $A_{\alpha}^k$ ) and B6 ( $A_{\beta}^b$ - $A_{\alpha}^b$ ), respectively. (For simplicity, the possible existence of two additional subgroups of cells restricted by *trans*-associated  $A_{\beta}^b$ - $A_{\alpha}^k$  and  $A_{\beta}^k$ - $A_{\alpha}^b$  molecules will not be considered.) The other two T cell subgroups are restricted by two sets of A/E hybrid molecules, i.e.,  $E_{\beta}^k$ - $E_{\alpha}^k$  and  $E_{\beta}^b$ - $E_{\alpha}^k$ . (It will be presumed that the apparent nonexistence of the  $E_{\alpha}^b$  chain precludes the possible existence of another two subgroups of T cells restricted by  $E_{\beta}^k$ - $E_{\alpha}^b$  and  $E_{\beta}^b$ - $E_{\alpha}^b$ , respectively.)

(b) The activity of broad spectrum anti- $I-A^k$  (A.TH anti-A.TL) antiserum has specificity for two sets of molecules, i.e.,  $A_{\beta}^k$ - $A_{\alpha}^k$  ( $I-A^k$ ) and  $E_{\beta}^k$ - $E_{\alpha}^k$  ( $I-A/E^k$ ); the activity for  $I-A/E^k$  is presumably directed to the  $E_{\alpha}^k$  chain because A.TH anti-A.TL antiserum has high activity for B10.A(5R) (*bbkkddd*) B cells (Materials and Methods).<sup>3</sup> When injected in vivo, the anti- $I-A^k$  and anti- $I-A/E^k$  antibodies bind to the corresponding molecules on macrophages and block the activation of T cells that recognize antigen in association with these molecules. Thus, A.TH anti-A.TL antiserum blocks selection of (i) the  $I-A^k$ -restricted T cells (which can interact with either B10.BR or B10.A[4R] B cells) and (ii) the  $I-A/E^k$ -restricted T cells (which interact only with B10.BR B cells and not with B10.A[4R] B cells). The minimal activity of A.TH anti-A.TL antiserum for  $I-A^b$  (B10) B cells does not demonstrably impair activation of the  $I-A^b$ -restricted T cells, i.e., cells that collaborate with B10 B cells. In contrast to A.TH anti-A.TL antiserum, monoclonal anti- $I-A^k$  antibodies block activation of only the  $I-A^k$ -restricted T cell group and not the  $I-A/E^k$ -restricted T cells.

Based on these assumptions, the data can be explained as follows (Table VIII):

TABLE VIII  
Interpretation of Results in Terms of T Cell Restriction by A/E Hybrid Molecules

Antibody added during selection	Host for selection	Specificity of selected T cells				Response with B cells		
		$I-A^k$	$I-A^b$	$E_{\beta}^k$ - $E_{\alpha}^k$	$(E_{\beta}^b$ - $E_{\alpha}^k)^*$	B10.A(4R) ( <i>kb</i> ) <sup>‡</sup> 1. $I-A^k$	B10.BR ( <i>kk</i> ) 1. $I-A^k$ 2. $E_{\beta}^k$ - $E_{\alpha}^k$	B10( <i>bb</i> ) 1. $I-A^b$
No serum	(CBA $\times$ B6) $F_1$	+	+	+	(+)	+	+	+
A.TH anti-A.TL (anti- $I-A^kE^k$ ) <sup>§</sup>	(CBA $\times$ B6) $F_1$	-	+	-	(-)	-	-	+
Anti- $I-A^k$	(CBA $\times$ B6) $F_1$	-	+	+	(+)	-	+	+
No serum	B10.A(4R)	+	-	-	(-)	+	+	-
Anti- $I-A^k$	B10.A(4R)	-	-	-	(-)	-	-	-

\* Hypothetical only.

<sup>‡</sup>  $I-A$ ,  $I-E$ .

<sup>§</sup> This antiserum could also have specificity for other *I*-region determinants.

<sup>3</sup> If A.TH anti-A.TL antiserum does have specificity for the  $E_{\alpha}^k$  chain, this antiserum presumably would also have specificity for  $E_{\beta}^b$ - $E_{\alpha}^k$  molecules.

*Selection in (CBA × B6)F<sub>1</sub> Mice.* With selection of (CBA × B6)F<sub>1</sub> T cells to SRC in irradiated F<sub>1</sub> mice in the presence of A.TH anti-A.TL antiserum, inhibition of selection of both the I-A<sup>k</sup>- and I-A/E<sup>k</sup>-restricted T cells results in the expression of only low (unprimed) help for both B10.BR and B10.A(4R) B cells. Selection of the I-A<sup>b</sup>-restricted T cells is not inhibited and these cells give high levels of help for B10 B cells. With selection in the presence of monoclonal anti-I-A<sup>k</sup> antibody, blockade of the I-A<sup>k</sup>-restricted T cells results in a marked reduction in help for B10.A(4R) B cells. Activation of the I-A/E<sup>k</sup>-restricted T cells is not blocked, however, and these cells give good collaborative responses with I-A/E<sup>k</sup>-bearing B10.BR B cells but do not interact with B10.A(4R) B cells which lack I-A/E<sup>k</sup>.

*Selection in B10.A(4R) Mice.* Unlike (CBA × B6)F<sub>1</sub> mice, the antigen-presenting cells in B10.A(4R) mice express only a single set of restricting Ia molecules, i.e., I-A<sup>k</sup>. Hence, positive selection of F<sub>1</sub> T cells to SRC in B10.A(4R) mice is limited to the I-A<sup>k</sup>-restricted T cell subgroup; after selection, these cells provide help for either B10.BR or B10.A(4R) B cells but not for B10 B cells. Blocking the selection of these T cells with anti-I-A<sup>k</sup> antibody (either A.TH anti-A.TL antiserum or monoclonal anti-I-A<sup>k</sup> antibody) thus inhibits help for both B10.BR and B10.A(4R) B cells.

This explanation is strictly a working hypothesis, and it is worth emphasizing that the data do not rule out the possible involvement of other *I*-region genes, e.g., those situated in the *I-B* or *I-C* subregions. To test the above hypothesis, the simplest approach would be to examine the blocking activity of monoclonal anti-E<sup>k</sup> antibodies,<sup>4</sup> as yet, we do not have access to such a reagent. As an alternative to using monoclonal antibodies, the technique of inducing negative selection of T cells to SRC in allogeneic irradiated mice (38) provides a useful approach. For example, in the case of CBA (*I-A<sup>k</sup>, I-E<sup>k</sup>*) T cells, one would expect that removal of the I-A/E<sup>k</sup>-restricted subgroup of cells would not occur in selection hosts expressing either I-A<sup>k</sup> or I-E<sup>k</sup> alone (B10.A[4R] and B10.A[5R], respectively) but would occur in hosts expressing both determinants (CBA [*cis*] or [4R × 5R]F<sub>1</sub> [*trans*]). Preliminary experiments suggest that this is indeed the case (J. Sprent and B. Alpert. Unpublished data.).

Certain aspects of the present data require comment. First, it might seem surprising that positive selection of T cells in F<sub>1</sub> mice in the presence of anti-I-A<sup>k</sup> antibody did not cause a detectable reduction in T cell help for B10.BR B cells, even when limiting-doses of T cells were used (Table IV). Thus, if the control response with B10.BR B cells reflected the combined action of both the I-A<sup>k</sup>- and I-A/E<sup>k</sup>-restricted T cell subgroups, inhibiting the activation of the I-A<sup>k</sup>-restricted T cells would be expected to cause an appreciable reduction in the B10.BR B cell response. The fact that there was no trace of a reduction could be explained in terms of a finite availability of T cell space. Thus, inhibiting the activation of one subgroup of T cells might increase the space available for positive selection (clonal expansion) of the other T cell subgroups. Testing this notion would be difficult.

Recently, Perry et al. (39) have reported that injection of microliter quantities of anti-Ia antibodies can inhibit the induction and expression of delayed-type hypersensitivity. Inhibition of T cell activation in the present system, by contrast, required prodigious quantities of anti-Ia antibody. In the case of A.TH anti-A.TL antiserum,

<sup>4</sup> An alternative approach would be to examine the blocking effects of A.TH anti-A.TL antiserum after appropriate absorption to remove activity to either I-A or I-E determinants. The prohibitive cost of this reagent has discouraged such an approach.

doses of <2 ml had minimal effects (whether smaller doses of monoclonal anti-I-A<sup>k</sup> antibody would have been effective was not studied). If the antibody does indeed act by binding to macrophages and thereby blocking T-macrophage interaction, it is perhaps not surprising that massive quantities were needed, particularly because much of the antibody was probably absorbed by other Ia-positive cells.

### Summary

To examine the role of Ia antigens in controlling T cell activation *in vivo*, unprimed (CBA × B6)F<sub>1</sub> (H-2<sup>k</sup> × H-2<sup>b</sup>) T cells were positively selected to sheep erythrocytes (SRC) for 5 d in irradiated F<sub>1</sub> mice in the presence of large doses of anti-Ia<sup>k</sup> antibody. With selection in the presence of broad-spectrum anti-Ia<sup>k</sup> antibody (A.TH anti-A.TL antiserum), the activated T cells were markedly reduced in their capacity to collaborate with either B10.BR (I-A<sup>k</sup> I-B<sup>k</sup> I-J<sup>k</sup> I-E<sup>k</sup> I-C<sup>k</sup>) (kkkkk) or B10.A(4R) (kbbbb) B cells but gave good helper responses with B10 (bbbb) and (B10 × B10.BR)F<sub>1</sub> B cells. Because there was no evidence for suppression, these findings were taken to imply that the anti-Ia<sup>k</sup> antibody bound to Ia determinants on radioresistant macrophagelike cells of F<sub>1</sub> host origin and blocked the activation of the Ia<sup>k</sup>-restricted subgroup of F<sub>1</sub> T cells but did not affect activation of the Ia<sup>b</sup>-restricted T cell subgroup.

Analogous experiments in which F<sub>1</sub> T cells were selected to SRC in F<sub>1</sub> mice in the presence of monoclonal anti-I-A<sup>k</sup> antibody gave different results. In this situation, the reduction in T cell help for Ia<sup>k</sup>-bearing B cells applied to B10.A(4R) B cells but not to B10.BR B cells. With selection of F<sub>1</sub> T cells in B10.A(4R) mice, by contrast, anti-I-A<sup>k</sup> antibody blocked T cell help for both B10.A(4R) and B10.BR B cells. These data suggested that genes telomeric to the *I-A* subregion were involved in controlling T cell activation and T-B collaboration. Because no evidence could be found that I-B through I-C determinants *per se* could act as restriction elements, the working hypothesis for the data is that Ia<sup>k</sup>-restricted T cells consist of two subgroups of cells: one subgroup is restricted by I-A-encoded molecules, whereas the other is restricted by I-A/E hybrid molecules encoded by two separated genes situated in the *I-A* and *I-E* subregions, respectively. The notion that A/E hybrid molecules serve as restriction elements is in line with the findings of other workers that these molecules can act as alloantigens and control responses to certain antigens under double *Ir* gene control.

The skillful technical assistance of Ms. K. Norris and the typing expertise of Ms. K. King and Ms. T. McKearn are gratefully acknowledged. I thank Mr. F. Symington for passaging the hybridoma line.

*Received for publication 13 May 1980 and in revised form 19 June 1980.*

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1010      BLOCKING T CELL FUNCTION IN VIVO WITH ANTI-Ia ANTIBODY

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